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Nucleotide sequence of the mitochondrial genes coding for $tRNA^{gly}_{GGR}$ and $tRNA^{val}_{GUR}$

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ABSTRACT

Yeast mitochondrial DNA-pBR322 recombinant DNA molecules known to contain tRNA genes from a tRNA rich region of the yeast genome were used as a source of DNA for restriction mapping and tRNA gene sequence analysis. We report here restriction maps of two segments of yeast mitochondrial DNA and the sequences of mitochondrial genes coding for $tRNA^{gly}_{GGR}$ and $tRNA^{val}_{GUR}$. Both genes are flanked by A + T rich DNA and neither has an intervening sequence nor codes for a 3' CCA end. The tRNA structures deduced from the genes have the usual cloverleaf structures and invariant nucleotides. This combination of DNA sequencing and restriction mapping has enabled us to determine that the $tRNA^{val}_{GUR}$ and a previously sequenced tRNA, the $tRNA^{phe}_{UUY}$ are transcribed from the same strand of DNA.

INTRODUCTION

Yeast mitochondrial DNA codes for mRNA, rRNA and tRNAs used in mitochondrial protein synthesis (1). Genetic (2,3) and physical (4,5) mapping techniques have demonstrated that about two-thirds of the tRNA genes are located in 20% of the 70 kilobase circular genome while the rest are dispersed. In order to determine the arrangement of the tRNA genes in more detail and to elucidate their fine structure we have been identifying, mapping and sequencing mitochondrial tRNA genes cloned in the E. coli plasmid pBR322. The identification of tRNA genes in the bank and their localization on the yeast genome by deletion mapping have been reported (6). Here we present the sequencing results of our studies on two tRNA genes from the tRNA rich region which is between the chloramphenicol and paramomycin resistance markers on yeast mitochondrial DNA. Mapping studies using one of these plasmids have enabled us to locate the $tRNA^{val}_{GUR}$ gene precisely on the genome and with respect to a phenylalanyl and threonyl tRNA gene. A comparison of the restriction map and tRNA gene sequences demonstrates that the $tRNA^{val}_{GUR}$ and $tRNA^{phe}_{UUY}$ (78) are transcribed from the same strand and the direction of transcription

is away from the chloramphenicol marker.

MATERIALS AND METHODS

Isolation of nucleic acids

Mitochondrial DNA was isolated from purified mitochondria as described previously (2). Mitochondrial tRNA was isolated from purified mitochondria (2) and labeled at the 3' end using [α-^{32}P]ATP and E. coli nucleotidyl transferase (6).

Restriction digests and filter hybridization

The various DNAs were digested with restriction enzymes according to procedures recommended by the commercial suppliers of the enzymes (New England Bio Labs or Bethesda Research Labs). Fragments were resolved on agarose-acrylamide gels (9), transferred to nitrocellulose by the method of Southern (10) and hybridized to radiolabeled tRNA for 16 hours at 60°C in Denhardt's buffer (11) containing 6 x SSC (SSC is 0.15 M NaCl, 0.015 M Na citrate). 2% agarose gels were used for the restriction mapping studies.

DNA sequence analysis

The 1020 base pair Hpa II fragment (Hpa 1020) was cut with Taq I and the resulting staggered 3' ends were labeled using [α-^{32}P]dCTP (Amersham) and E. coli DNA polymerase I (Boehringer Mannheim) (12).

The 1420 base pair Hpa II fragment (Hpa 1420) was cut with Taq I and the resulting staggered 3' ends were labeled using [α-^{32}P]dCTP. Overlaps in the sequencing of this gene were obtained. One of the fragments from an Alu I digestion of the 1420 fragment was labeled using polynucleotide kinase and [γ-^{32}P]ATP and sequenced. In addition, the nucleotide sequence around the Hinf I site was determined in both directions following labeling of the 3' ends of the Hinf I site with [α-^{32}P]dATP and DNA polymerase.

The sequence between 2 and 25 nucleotides from the labeled ends was determined by fractionation of the cleavage products on ultrathin 20% polyacrylamide gels (39 x 39 cm) containing 7 M urea. The sequence between 25 and 300 nucleotides was determined by fractionation of the cleavage products on ultrathin 6% polyacrylamide gels (87 x 25 cm) containing 7 M urea.

RESULTS

Identification of fragments that contain tRNA genes in mitochondrial DNA-pBR322 recombinants

Native mitochondrial DNA and mitochondrial DNA-pBR322 recombinant DNA

was digested with Hpa II, electrophoresed on acrylamide-agarose composite gels, transferred to nitrocellulose and hybridized with bulk labeled mitochondrial tRNA (Figure 1). Using this technique we identified a 1020 base pair fragment in pYm 318 and two fragments, 1420 and 680 base pairs in pYm 424 that hybridize mitochondrial tRNA. Fragments of the same sizes were also observed in the native mitochondrial DNA. The sequence of the 680 fragment revealed that it contained one tRNA gene encoding a $tRNA^{phe}_{UUY}$ (8).

Restriction map of plasmids pYm 318 and pYm 424

Plasmid pYm 318 contains a 2100 base pair insert. We have located the restriction sites for Alu I, Hinf I and Hpa II within the insert (Figure 2). Since this insert was not cleaved by any of the restriction enzymes whose sites have been mapped on the wild type genome (13) we could not orient it with respect to other known physical markers.

Plasmid pYm 424 contains a 4500 base pair insert. We have located the restriction sites for Alu I, Ava II, Hinc II, Hinf I, Hpa II and Xba I in this insert (Figure 3). The Xba I and Hinc II site are separated by 1040 base pairs. The only Hinc II and Xba I site in the wild type genome that are this close together and between the chloramphenicol and paramomycin loci are located at 19-20 map units (13). A comparison of genetic mapping (2,3) and

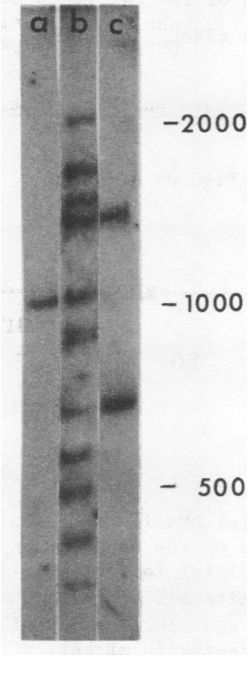


Fig. 1. Autoradiogram of Hpa II digests of (a) pYm 318, (b) native D273-10B mitochondrial DNA, (c) pYm 424 that have been run on 2.5% acrylamide, 0.5% agarose gels, transferred to nitrocellulose and hybridized with ^{32}P-mitochondrial tRNA. The numbers at the side refer to base pairs.

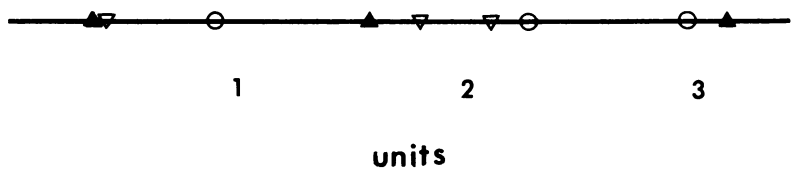


Fig. 2. Restriction enzyme site map of the mitochondrial DNA insert contained in pYm 318. Each unit corresponds to 700 base pairs in the wild type genome. Alu I (▽), Hinf I (▲), Hpa II (o). The tRNA gene is located at the Hinf I site in the center of the map.

our fine structure restriction mapping demonstrates that these two sites are in the opposite orientation to that reported previously (13), i.e., the Xba I site is closer to the chloramphenical marker than is the Hinc II site. As shown in figure 3 mapping of the restriction enzyme sites located within the mitochondrial DNA insert of plasmid pYm 424 reveals that the genes coding for $tRNA^{val}_{GUR}$ and $tRNA^{phe}_{UUY}$ are located about 1200 base pairs apart on adjacent Hpa II fragments. Since the orientation of these fragments can be determined by analysis of the location of internal Hinf I, Ava II and Xba I restriction sites, and because the orientation of the tRNA genes has been determined by DNA sequence analysis (7,8, this work), the orientation of the two genes to each other can be determined. The coding sequences for $tRNA^{phe}_{UUY}$ and $tRNA^{val}_{GUR}$ are located on the same strand.

Sequence analysis of tRNA genes in the pYm 424 Hpa 1420 base pair fragment and the pYm 318 Hpa 1020 base pair fragment

The results of the DNA sequencing can be seen in Figures 4 and 5.

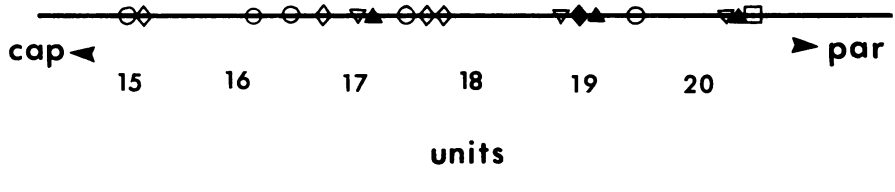


Fig. 3. Restriction enzyme site map of the mitochondrial DNA insert contained in pYm 424. Each unit corresponds to 700 base pairs on the map of Morimoto and Rabinowitz (13). Alu I (▽), Ava II (◇), Hinf I (▲), Hinc II (□), Hpa II (o), Xba I (◆). The $tRNA^{val}_{GUR}$ is located at 19 units and contains a Hinf I site while the $tRNA^{phe}_{UUY}$ is located at 17.2 units and contains a Hinf I site. Cap and par refer to the chloramphenicol and paramomycin marker.

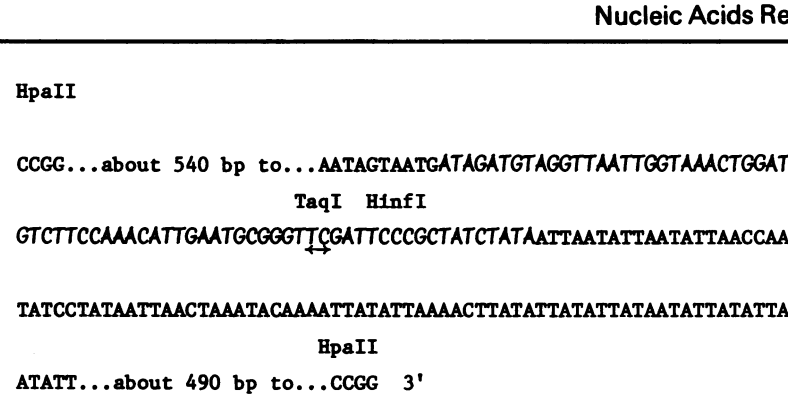


Fig. 4. The sequence of the yeast mitochondrial $tRNA^{gly}_{GGR}$ gene. Only the non-coding strand is presented. The arrows indicate the directions of sequencing away from the labeled Taq I site. The tRNA sequence is in italics.

Figure 4 summarizes the results of sequencing a portion of the Hpa 1020 fragment. The sequence 5' to the Taq I site was obtained directly as written while the sequence 3' to the Taq I site was obtained from the sequence of the complimentary strand. When the region of the fragment that codes for the tRNA is folded into a cloverleaf structure the anticodon is 5' UCC 3'. Therefore, this gene codes for a mitochondrial $tRNA^{gly}_{GGR}$. Figure 6a shows the cloverleaf structure of the $tRNA^{gly}_{GGR}$ that we have deduced from the DNA sequence.

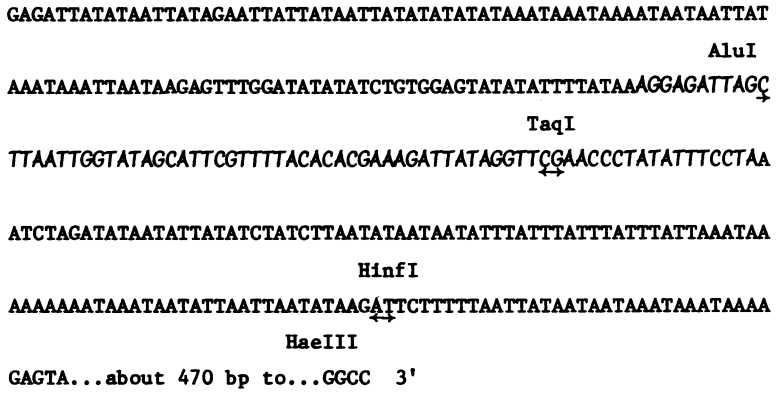
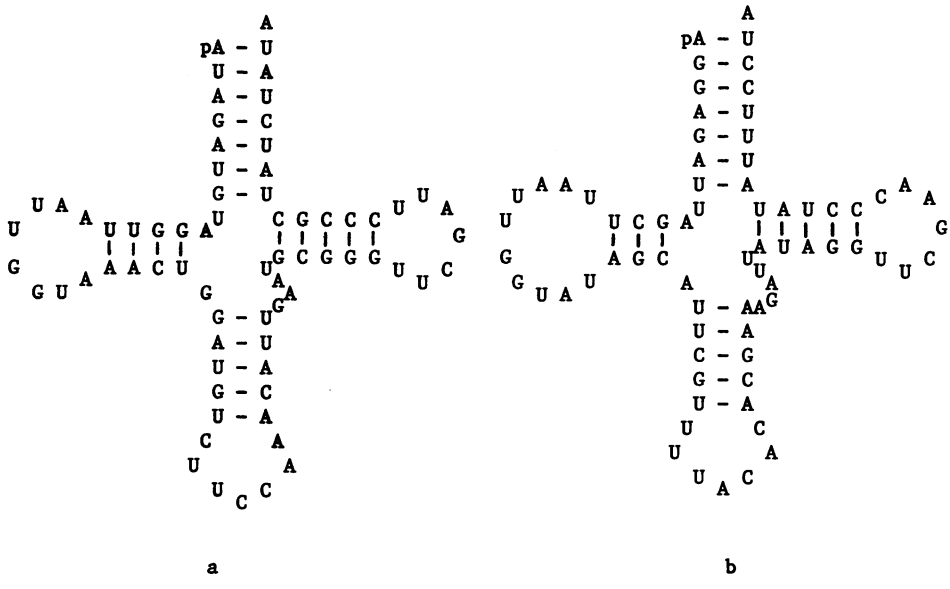


Fig. 5. The sequence of the yeast mitochondrial $tRNA^{val}_{GUR}$ gene. Only the non-coding strand is presented. The arrows indicate the directions of sequencing away from the labeled ends.



Figs. 6a & b. Cloverleaf models for yeast mitochondrial $tRNA^{gly}_{GGR}$ (a) and $tRNA^{val}_{GUR}$ (b).

Figure 5 summarizes the results of sequencing a portion of the Hpa II 1420 fragment. Only the non-coding strand is shown. When the region of the fragment that corresponds to this tRNA sequence is folded into a cloverleaf the anticodon is 5' UAC 3' and this corresponds to a $tRNA^{val}_{GUR}$. The tRNA structure deduced from this sequence is shown in Figure 6b.

DISCUSSION

Sequence analysis of mitochondrial tRNA genes yields information about the tRNA structures themselves. A number of yeast mitochondrial tRNA genes have been sequenced (6,7,8,14,15,16) and some of the tRNAs coded by these genes contain features not previously found in tRNAs. The $tRNA^{gly}_{GGR}$ and $tRNA^{val}_{GUR}$ are not, however, atypical in secondary structure. Nor are they atypical in their invariant nucleotide characteristics as elaborated in (17). Both have a U in position 8 and an A in position 14. There is a GG sequence in the D loop so they can pair with $U_{55}C_{56}$ and form the teritiary structure dictated by this pairing. Both have a U at position 33, a G at position 53,

a C at position 56 and a TTC sequence in the TΨC loop. It is likely that the tRNAs will have Ψ at position 55 but direct sequencing of the tRNAs will be necessary to ascertain this. A consideration of semi-invariant positions in these tRNAs shows that both have purines at positions 9, 15 and 57. Both have pyrimidines at the position before the TΨC stem begins and at the end of the TΨC loop. The $tRNA^{gly}_{GGR}$ does not have a pyrimidine at position 11 and the $tRNA^{val}_{GUR}$ does not have a pyrimidine in the first position of the anticodon loop.

Genes encoding yeast mitochondrial tRNAs that have anticodons corresponding to arginine (16), cysteine (14), glycine (7, this work), histidine (14), phenylalanine (8), serine (7,8,16), threonine (15), and valine (7,15, this work), have now been sequenced. None of them encode a 3' CCA end nor do any contain intervening sequences such as those found in yeast nuclear tRNA genes. All of these genes are surrounded by ver A + T rich DNA but there are no obvious specific base sequences within the flanking regions that all the tRNA genes share. It may be that signals for initiation and termination of transcription are close to the structural genes but the lack of base sequence homology between the different tRNA genes makes it impossible to predict their exact locations. Alternatively, initiation and termination in mitochondria might require A + T rich regions but not specific sequences. Yet another alternative would be that initiation and termination occur far from the structural genes.

After this work was completed, Li and Tzagaloff (15) reported the location sequence and orientation of the $tRNA^{thr}_{CUR}$ and $tRNA^{val}_{GUR}$ gene on DNA isolated from petite mutants. A comparison of their restriction map with the map in figure 3 shows that the $tRNA^{thr}_{CUR}$ gene is between the $tRNA^{phe}_{UUY}$ and $tRNA^{val}_{GUR}$ gene on the 1420 base pair Hpa II fragment. The $tRNA^{thr}_{CUR}$ gene is transcribed from the opposite strand from which the $tRNA^{val}_{GUR}$ (15) and $tRNA^{phe}_{UUY}$ genes are transcribed. If the $tRNA^{phe}_{UUY}$ and the $tRNA^{val}_{GUR}$ are derived from the same transcriptional unit, then both strands of the $tRNA^{thr}_{CUR}$ gene must be transcribed. Alternatively, all three genes may be transcribed independently.

The wild type strain from which we obtained mitochondrial DNA for cloning is the same strain as that from which Li and Tzagaloff obtained their petites. A comparison of the sequences from the two laboratories shows only 4 differences in 310 base pairs, all in the A + T rich spacer regions. The ease with which cloned mitochondrial DNA can be isolated will facilitate future studies on the transcription and organization of this unique eukaryotic genome.

ACKNOWLEDGEMENTS

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